

SPECIAL ARTICLE

Aluminum as an adjuvant in Crohn's disease induction

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Alum ($\text{AlK}(\text{SO}_4)_2$) is an adjuvant commonly utilized in vaccines, and is a ubiquitous element used extensively in contemporary life. Food, air, water, waste, the earth's surface, and pharmaceuticals all represent pathways of aluminum (Al) exposure. Crohn's disease (CD) is a chronic relapsing intestinal inflammation in genetically susceptible individuals and is caused by yet unidentified environmental factors. Al is a potential factor for the induction of inflammation in CD, and its immune activities share many characteristics with the immune pathology of CD: many luminal bacterial or dietary compounds can be adsorbed to the metal surface and induce Th1 profile cytokines, shared cytokines/chemokines, co-stimulatory molecules, and intracellular pathways and stress-related molecular expression enhancement, affecting intestinal microbiota, transmural granuloma formation, and colitis induction in an animal CD model. The inflammasome plays a central role in Al mode of action and in CD pathophysiology. It is suggested that Al adjuvant activity can fit between the aberrations of innate and adaptive immune responses occurring in CD. The CD mucosa is confronted with numerous inappropriate bacterial components adsorbed on the Al compound surface, constituting a pro-inflammatory supra-adjuvant. Al fits the diagnostic criteria of the newly described autoimmune/inflammatory syndrome induced by adjuvants. If a cause and effect relationship can be established, the consequences will greatly impact public health and CD prevention and management. *Lupus* (2012) **21**, 231–238.

Key words: adjuvant; aluminum; Crohn's disease; environmental factor; intestine; pathogenesis

Introduction

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract that presents clinically most often with diarrhea, abdominal pain, and weight loss. Any segment of the gastrointestinal tract can be affected in a discontinuous pattern, and there are many extraintestinal manifestations such as hepatitis, cholangitis, arthritis, erythema nodosum, and iritis. CD affects around 50–200 patients per 100,000 individuals in Western countries. The symptoms of CD vary depending on the location of the inflammation. Growth impairment is often seen even before diagnosis. Perianal fissures, fistulae, and abscesses occur. One of the pathological hallmarks of the disease is transmural granulomatous inflammation. CD is an immunologically distinct entity associated with a Th1

T-cell-mediated response including enhanced production of interferon-gamma and tumor necrosis factor-alpha, governed by interleukin (IL)-12 and IL-23. IL-1 β , IL-18, and IL-21 are also involved.

The precise etiology of CD is not well understood, but is thought to be related to a combination of genetic and environmental factors that impact on normal host–microbe interactions. In fact, loss of tolerance to enteric microbiota is suspected. An as yet incompletely defined subset of intestinal bacteria is required to stimulate chronic, immune-mediated intestinal inflammation in CD, and intestinal microbiota composition is altered to create a dysbiotic relationship of protective versus aggressive bacteria.¹

Environment and CD

Environmental factors unquestionably play a major role in the pathogenesis of CD,² smoking, breast-feeding, early infection, dietary factors, and higher economical status having been suggested.

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The intestinal commensal bacterial flora is, however, the environmental factor most frequently implicated,³ including *Mycobacterium avium paratuberculosis*, *Escherichia coli* and *Helicobacter* sp. Human studies further strengthen this association: (i) CD develops in areas of high bacterial concentration such as the terminal ileum and colon; (ii) diversion of the fecal stream prevents intestinal inflammation; (iii) antibiotics and probiotics are beneficial; (iv) antibodies against bacterial components have been detected;⁴ and (v) lymphocytes from patients are reactive to fecal antigens. Furthermore, experimental colitis does not develop under germ-free (sterile) conditions and lymphocytes that are reactive to bacterial antigens induce colitis after passive transfer to immunologically naïve recipients.

Among potential dietary environmental agents, however, none has yet emerged as a favorite. Offending dietary constituents need not to be conventional foodstuffs. Additives, preservatives or even micro- or nanoparticle contaminants are plausible alternatives.^{5–7} Al was first suggested, in 1987, by Ganrot as a possible etiologic agent in CD.⁸ Perl et al. extended the hypothesis in regard to the induction of CD to other trace elements in the interplay between bacteria and metals.⁹ The present review is based on an extensive review of CD carried out in 2007,¹⁰ and is intended to summarize and update the scientific community on the topic.

Human exposure to aluminum

Aluminum is a ubiquitous element used extensively in contemporary life.¹⁰ A wealth of research now suggests that it is toxic to plants, aquatic animals, and humans. Overall, the daily human intake of dietary inorganic microparticles in the UK has been estimated at around 40 mg, which equates to a staggering daily exposure of 10^{12-14} particles per person.¹¹ Human exposure can arise from the following sources.

Environmental

Natural Al occurs in the soil and makes up about 8% of the earth's surface. Higher concentrations are found in waste areas, coal combustion, the Al mining and melting industry, and in flux of dust from ores and rock material. In the atmosphere, it is mainly found as aluminum silicates, their background levels ranging from 0.005 to 0.180 mg/m³. Urban water carries higher levels of Al than natural sources. An increasing amount of AL is leached continuously by acid rain and contributes significantly to environmental input. Worldwide, it has

been estimated that 40% of arable soils and 70% of cultivable lands are sufficiently acidic to have an Al toxicity problem.

Dietary

When the soil pH is <4.5–5.0, Al is solubilized in the soil water and absorbed by plant roots. The following nutrients are rich in Al: food additives, purified drinking water, processed cheese, non-dairy creamers, powdered milks or food, baking powder, cake mixes, pancakes, sugars, soy-based milk products, chewing gum, instant pot savory snacks (rice and noodles), vending machine drinks, and icing sugar.¹¹ Leaching of Al from beverage cans and cookware also occurs; it has been estimated that about 20% of human daily intake of Al comes from cooking utensils. The mean Al intake by adult males and females is 10 and 7 mg/day, respectively.

Iatrogenic

The following all contain Al: dialysates, phosphate binders, antacids, intravenous solutions, urinary bladder irrigation fluids, bone and dental cements, buffered aspirins, antidiarrheal and antihemorrhoidal medications, vaccine adjuvants, nutraceuticals, and toothpaste.

Occupational

Exposure to Al dusts and fumes are among occupational hazards of workers in the Al refining and metal industry, printing and publishing, and automotive dealerships and services.

Aluminum and the gastrointestinal tract

Human studies

The first study to describe exogenous pigment in human Peyer's patches was that by Shepherd and colleagues in 1987,¹² where all normal and diseased samples of the small bowel of individuals above the age of 6 years – but none from those <6 years – were positive. On analysis, the macrophages in the pigmented Peyer's patches contained Al and silicon, diffusely spread throughout the cytoplasm, around dilated submucosal lymphatics, and in mesenteric lymph nodes. Following on from that, Urbanski et al. documented a black pigment within macrophages in the lamina propria and submucosa of human Peyer's patches.¹³ Most of the particles were predominantly Al and magnesium-rich silicates and were considered of exogenous origin. Powell et al. then extended our knowledge on the

physicochemical structure of these pigments.¹⁴ Laser scanning and electron microscopy showed macrophage phagolysosomes in human gut-associated lymphoid tissue, loaded with three types of microparticles, one of them being aluminosilicates of <100–400 nm in length. These particles were submicron sized and highly resistant to chemical breakdown. After mucosal uptake, they survived gastrointestinal digestion and cellular processing. Human exposure to aluminosilicates and titanium dioxide is chiefly through food additives, pharmaceuticals, and toothpaste, particles being predominantly deposited in the basal phagocytes of Peyer's patches.¹⁵ These authors suggested this cellular pigment, derived from the environment, to comprise inert inorganic microparticles that in susceptible individuals cause chronic latent granulomatous inflammation.

Animal studies

In animal experiments, dogs fed on a daily ration of finely divided sand or talcum powder developed intestinal inflammation with pronounced lymphedema and pathological features of regional enteritis.¹⁶ A cluster of six lethal equine granulomatous enteritis cases linked to Al were described by Fogarty et al.,¹⁷ where tissue Al concentrations in the intestine of all horses were elevated and many histological similarities to human CD were noted. Chemical analysis demonstrated concentrated Al within intestinal wall microorganisms suggesting microorganism–Al interaction associated with the equine condition.⁹ Recently, we studied the effect of dietary Al in specific pathogen-free, microbiota-induced colitis in an IL-10 knockout mouse model,¹⁸ where increased Al lactate concentrations of up to 200 μ M in the drinking water stimulated T-cell proliferation and interferon secretion by splenocytes. The drinking of water containing higher levels of Al worsened colitis, as documented by significantly increased colonic histological scores. Colonic strip IL-12 secretion increased with higher Al intake. Of note, higher dietary Al induced pink *E. coli* colonies as cultured on MacConkey agar plates, suggesting that Al influences intestinal bacterial ecologic composition. This represents the first causal relationship between environmental Al stimulating intestinal and systemic immune responses, in vitro and enhancing colitis, in vivo, in a colitic animal model.

Gastrointestinal microparticle translocation

In a recent review, Powell et al.⁷ described potential routes for translocation of micro- or nanoparticles

across the gastrointestinal mucosa: (i) transepithelial pathway, for very small particles of diameter > 100 nm; (ii) transcytosis, representing specialized M-cell epithelial uptake, for mainly large nanoparticles (20–100 nm) or microparticles (100–500 nm); (iii) persorption, whereby the gape in epithelial integrity created by shaded enterocytes, at the villous tip, is at the origin of increased permeability to large particles of 100–500 nm diameter. Alternatively, the increased epithelial apoptosis described in CD might potentiate the epithelial gape;¹⁹ and (iv) paracellular transcytosis across tight junctions in the epithelial layer. A break in this highly functional machinery can occur through the action of drugs, epithelial cell metabolites, dietary solutions (glucose), or calcium chelators (citrate), and as a result of certain diseases such as CD or celiac disease.^{20,21} Recently, an imbalance was suggested between absorption of essential nutrients and prevention of entry of harmful luminal contents. Increased permeability, via both the transcellular and paracellular routes and increased apoptosis of epithelial cells, occurs in CD.²¹ The intracellular NOD-like receptor Nlrp3 has now emerged as a crucial regulator of intestinal homeostasis. Most recently, defective Nlrp3 inflammasome signaling in the gut, contributing to CD through increased permeability across the epithelial barrier and the induction of compromised immune responses against invading commensals, was described.²² Since alum adjuvants operate through the Nlrp3 pathway of the inflammasome,²³ there is a possibility in CD that Al complexes bypass the defective Nlrp3 system inflammasome, thus increasing the immunogenicity of harmful intestinal bacterial products, resulting in enhanced inflammation.

In summary, barrier dysfunction in inflammatory bowel disease causes mucosal inflammation secondary to uptake of luminal constituents. Barrier abnormalities, such as epithelial tight junction disruption, increased epithelial apoptosis, gross mucosal lesions, epithelial restitution arrest, or defective functioning of Peyer's patches or M epithelium^{24,25} may operate in concert to increase the toxicity of nano- or microparticles containing Al, thus inducing or aggravating CD.

Aluminum and CD

A major contribution to the topic on the relationship between CD and microparticles was made by Powell and co-workers in the UK,^{7,11,14,15,26–33}

while Perl et al. progressed the hypothesis on bacterial-metal interaction in the etiology of CD.⁹

In the Western diet a substantial level of ultrafine particles are ingested per person every day, and these microparticles adsorb luminal constituents and are taken up by the intestinal lymphoid tissue. Based upon their surface adsorptive capacity, organic luminal components can conjugate and the resultant complex may mimic bacterial pathogenicity towards cells.^{15,26} The addition of a food additive, such as titanium dioxide particles to a standard cell culture medium enhanced peripheral cell responses to lipopolysaccharide (LPS), resulting in pro-inflammatory signaling (release of IL-8 and TNF α and reduced secretion of TGF- β 1).³³ This effect was enhanced when calcium was added to the medium. Powell's group showed a LPS-calcium-microparticle conjugate to be readily taken up by phagocytic cells and to induce a pro-inflammatory reaction, as evidenced by the secretion of IL-1 β .²⁷ LPS is able to trigger pro-IL-1 β production, while calcium phosphate alone or as

part of the calcium phosphate-TiO₂ aggregate promotes caspase 1 maturation, resulting in cleavage of pro-IL-1 β to mature pro-IL-1 β .^{26,28} Thus, the effects of IL-1 β secretion are genuinely synergistic between the components of the conjugate.⁷ It is worthwhile mentioning that Al is known to induce IL-1 secretion from human peripheral blood monocytes³⁴ and that levels of IL-1 β , IL-18, and caspase 1 are increased in CD due to the defective autophagy implicated in this disease. Autophagy, a bulk degradation system that converts cytoplasmic constituents into autolysosomes and controls the degradation of long-lasting proteins, insoluble protein aggregates, and invading microbes, does not function appropriately in CD patients.³⁵ More importantly, loss of the autophagy protein Atg16L1 enhanced LPS-induced IL-1 β production and increased susceptibility to dextran sulphate sodium-induced acute colitis, which was alleviated by the injection of anti-IL-1 β and -IL-18 antibodies.³⁵ It appears that the inflammation/autophagy mechanism is activated by Al

Table 1 Shared parameters between aluminum and Crohn's disease*

<i>Aluminum</i>	<i>Crohn's disease</i>
1. Epidemiology	
Increased exposure in industrial societies	Increased prevalence in industrial area
2. Genetic influence	
Genetic influence on Al intestinal permeability	Genetically increased intestinal permeability
Al-induced pathology is genetically determined	CD is genetically determined
Induces DNA helical transition and damage	Increased DNA damage
3. Immunological aspects	
IL-12 overcomes Al TH2 polarization	IL-12 induces TH ₁ differentiation
Alum + IL-12 induces TH ₁ response	TH ₁ class disease
Alum + cytokines improves TH ₁ effectiveness	Increased TH ₁ cytokine profile
Induces macrophage differentiation	Increased mature dendritic cells
Macrophage cytotoxicity is Al particle size dependent	Decreased macrophage function
Promotes humoral immune response	Expanded B-cell population
Triggers inflammasome: IL-1 β , IL-18, caspase 1 secretion	Caspase 1, IL-1 β , IL-18 are increased
Transepithelial transport	Increased mucosal permeability
Al-calcium-bacterial antigen complex formation	Disrupted bacterial tolerance
4. Inflammatory profile	
Pro-inflammatory gene expression	Classical inflammatory state
Pro-oxidant activity	Increased oxidative stress and pro-oxidants
Induces stress-related gene expression	Increased HSP70, cox-2
Induces TNF- α release	TNF drives inflammation
Induces IL-6 release	Increased IL-6, IL-2r, MIP1- α
Induces chemokines and co-stimulatory molecules	Similar induction
Activates NFB, HIF-1	Deregulated NFB, activated HIF-1
Induction of endogenous danger signal uric acid	Alloperinol is clinically beneficial
5. Iron-transferrin relationship	
Al competes with Fe at transferrin receptors	Decreased iron intake and body stores
Increased Al uptake by rich transferrin receptor cells	Increased free transferrin receptor
Tissue Al uptake of TfC ₂ genetically determined	Iron enterocyte delivery is defective

*adapted from Lerner¹⁰.

complexes and that the latter is defective in CD, resulting in a surge in levels of pro-inflammatory cytokines.

Assessment of inorganic microparticle intake in CD patients in a UK study showed no differences compared with controls.³² However, a diet low in calcium and exogenous microparticles appeared to alleviate the symptoms of ileal CD, with significant improvement in the CD activity index.¹¹ If exposure to microparticles is associated in CD with intestinal inflammation, excess intake can be ruled out as a possible problem. After identification of foods containing low levels of microparticles, the same group assessed the impact in CD of a diet low in microparticles. In their preliminary study, the CD activity index improved significantly.³⁶ However, four years later, the same group conducted an adequately powered and carefully carried out dietary trial in patients with active CD. No evidence was seen to indicate that reduction in dietary microparticle intake achieved remission in those patients.³⁷ Whether dietary microparticles may be involved in the initial triggering or recurrence of CD in genetically susceptible subjects, as a cause and effect relationship, is a challenge for future investigations.

Table 1 summarizes the epidemiological, genetic, immunological, inflammatory, and iron factors linking AI and CD.

Discussion

The cellular and molecular mechanisms of induction of immune responses by aluminum adjuvants have recently been unraveled.²³ AI salts activate dendritic cells, monocytes, and macrophages with

enhanced expression of adhesion molecules CD45 and CD58 and co-stimulatory molecules CD40 and CD86, which are crucial in T-cell activation. The chemokines CCL2, CCL3, CCL4, and CCL8 are induced, thus mediating the recruitment of inflammatory cells at the site of vaccination. A parallel activation of the inflammatory cascade occurs in the inflamed intestinal mucosa in CD.^{38–42}

An interesting intersection between the AI mode of action and defective pathways in CD is the mechanism of inflammasome/autophagy.^{23,25} At first glance, the two oppose each other: alum is an activator of the inflammasome, while in CD this fails. On a broader scale over multiple steps and cellular events, inflammatory pathways, pro-inflammatory cytokines/chemokines/co-stimulatory molecules, endogenous danger signals, Th1 activation, and granuloma formation are common to both AI and CD (Table 1). It appears that the outcome of the two conditions is comparable: both show an induced inflammatory cascade enhanced by activation of IL-1 β , IL-18, and caspase 1. In both there is tissue damage, enhanced cellular generation of reactive oxygen species, transepithelial transport of foreign molecules, and activation of the transcription factor NF κ B, leading to pro-inflammatory signaling. Recent evidence is emerging to support the important intersection between three fundamental cell biologic pathways in CD pathogenesis: defective autophagy, failure of intracellular bacterial sensing, and unfolded protein accumulation response.⁴³ Adding the malfunction of Panet cells in response to luminal environmental factors, including microbes, the result is altered intestinal epithelial cell function and breakdown in tolerance toward gastrointestinal microbiota.

Table 2 Potential modes of action of AI particles in the pathogenesis of Crohn's disease

Aluminum	Crohn's disease
1. Enhances cellular generation of reactive oxygen species (ROS)	Increased ROS intestinal damage
2. Triggers inflammasome: IL-1 β , IL-18, caspase 1 secretion	Caspase 1, IL-1 β , IL-18 are increased
3. Adjuvant activity as surface-adsorbed molecules	Bacterial constituent intolerance
4. Th2 to Th1 shift	Th1 disease
5. Al-calcium-bacterial antigen complex	Impeded bacterial tolerance
6. Transepithelial transport and immunogenicity	Increased mucosal permeability
7. Increases susceptibility to tuberculosis	Increased mycobacterium virulence
8. Alters membrane lipid structure of activated DC, resulting in antigen uptake, enhanced binding with CD4(+)	Activated pro-inflammatory DC
9. Activates Nalp3 inflammasome to produce IL-1 β	Activated CD4(+)
	Due to defective NOD2, Nalp3 is over activated by bacterial component
10. Increases apoptosis	Increased enterocyte apoptosis
11. Increases multiple pro-inflammatory cytokines, hemokines, and co-stimulatory molecules	As for aluminum

DC, dendritic cell.

The CD mucosa is confronted with numerous inappropriate bacterial components.

On the other hand, Al/Alum/Al complexes are adjuvants that potentiate the immunogenicity of various dwelt bacterial antigens. One could visualize an ideal mucosal condition where this adjuvant exerts an innate and adaptive immune capacity,⁴⁴ facing the undwelt surge of pathogenic bacterial products, resulting in enhanced intestinal inflammatory cascade in CD patients. In fact, intestinal microorganisms trigger an over-reactive immune response in CD, of which ASCA, OmpC, I2, CBir1, and the new anti-glycan are serological examples.^{4,45} It is suggested that Al adjuvant activity can fit between the aberrations of innate and adaptive immune response occurring in CD. Although it is now generally accepted that Al adjuvants stimulate TH2, it was noted that an Al(OH)₃-IL-12 complex induced a TH1 response, rather than a TH2 one. The TH1-promoting effect of this complex was greatly augmented by the co-administration of exogenous IL-18.⁴⁶

Table 2 lists further potential modes of action whereby Al compounds may contribute to the pathogenesis of CD.

One practical aspect of the present review is the policy of vaccination in CD. A combination of the current use of steroids, immune modulators, and biological therapy and malnutrition/dietary deficiencies places patients at high risk of infection and they should therefore be vaccinated.⁴⁷ Currently, Alum is commonly used worldwide as an adjuvant in human vaccines (HepB, DTaP, Hib, PCV7, HepA). Based on the potential risk of Al in regard to induction of CD, one should be cautious in this high-risk population and use alternative adjuvants.

Several case reports and epidemiological data strengthen the involvement of Al in autoimmunity induction. Metal chelation therapy in Al intoxication was beneficial in rheumatoid arthritis.⁴⁸ Alum was found to induce macrophagic myofasciitis, an autoimmune-related disease.⁴⁹ Positive relations exist between Al levels in drinking water and Alzheimer's disease.⁵⁰ Elevated urinary excretion of Al and iron was found in multiple sclerosis.⁵¹ Pulmonary sarcoid-like granulomatosis induced by Al was described.⁵²

There are sufficient data to support the fact that Al, as an environmental factor, should be placed in a wider context of the mosaic of autoimmunity.⁵³ The known association between infection, vaccines, adjuvants, and autoimmunity is increasing, on both a clinical basis and in research.⁵⁴⁻⁵⁷ Most recently, a new syndrome – autoimmune/inflammatory

syndrome induced by adjuvants (ASIA) – has been established, and even diagnostic criteria suggested,^{58,59} with adjuvants such as alum and infectious components playing an important role as triggers of the syndrome. They increase the local reaction to antigens resulting in inflammatory cascade activation, provide physical protection to the antigens, and aid in antigen translocation to regional lymph nodes or adsorb immunogenic compounds to create a harmful pro-inflammatory complex. Molecular mimicry, epitope spreading, or polyclonal activation were suggested as mechanisms in regard to bacterial theories in the adsorption to alum in the generation of autoimmunity. For many years, alum adjuvants were considered safe and wholly beneficial in the fight against severe infections. One cannot underestimate their input in the struggle to prevent infections. On the basis of our current knowledge and the potential of Al to induce autoimmunity – specifically in CD – it will be rewarding to invest skill and resources in developing a new generation of safer and efficient adjuvants.

In conclusion, multiple factors, both clinical, immunological and physical, render Al and its compounds at risk for induction of CD. These relations are associative and await more causative proof.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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